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Anaplasma phagocytophilum propagation is enhanced in human complement-containing medium

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Complement was originally discovered as a component of normal plasma that augments opsonisation of bacteria by antibodies with subsequent killing of the pathogen. Complement plays a crucial role in innate and adaptive immune responses to many blood-borne pathogens [1,2], and some pathogens subvert complement function to survive in the blood. Its role in *A. phagocytophilum* infection has not been explored.

We previously showed reduced phagocytosis by neutrophils infected by *A. phagocytophilum*. Reduced phagocytosis and opsonophagocytic receptor loss could allow *A. phagocytophilum* to avoid killing by either the neutrophil or by complement activation. Such changes could result from down-regulated expression or shedding of opsonophagocytic receptors on neutrophil surfaces, including various Fc receptors and complement receptors that bind opsonising ligands such as IgG, IgA, IgE, or complement components. This hypothesis was previously tested in our laboratory by assaying for the expression of these receptors after 24 h of *A. phagocytophilum* infection in human neutrophils. *A. phagocytophilum*-infected neutrophils had significantly reduced levels of surface-exposed receptors C1qRp, FcRI, FcRII and FcRIII 24 h after infection. The expression of CR1 was slightly reduced, and there was very low or no expression of FcRII and FcRI in all cells [3].

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The fact that *A. phagocytophilum* alters reactions involving components of the complement system raises a more important question: how does it survive in the presence of complement factors during bloodstream infection, where other bacteria are usually effectively removed? Because other bloodstream bacterial pathogens, such as *Borrelia burgdorferi* [4] and *Streptococcus pneumoniae* [5], have evolved towards mechanisms that preclude complement-mediated opsonisation, assembly and lysis, it suggests that *A. phagocytophilum* is also likely to have evolved towards such mechanisms. We therefore hypothesised that *A. phagocytophilum* evades complement-mediated degradation to ensure survival.

A. phagocytophilum growth in human AB serum (NHS) with known CH50 lytic activity was compared with its growth in other serum supplements. HL-60 cells were used directly or after acclimation to 5% heat-inactivated (HI) NHS for 2–4 weeks. Experimental conditions included infected HL-60 cells in fetal bovine serum (FBS), HI-FBS, NHS and HI-NHS. Experiments were performed at 2.0×10^5 cells/mL starting at concentrations with differing initial infection rates. Infections were allowed to propagate for 5 days or until 100% infection was achieved, and bacterial load was quantified by qPCR using the $\Delta\Delta$Ct method or by using a standard curve of infected cells, and by light microscopy.

Without adaptation to NHS, *A. phagocytophilum* grew equally well in HI-NHS as in NHS, but neither condition supported growth as well as FBS (not shown). Once adapted to NHS, *A. phagocytophilum* grew more rapidly (maximum infection by day 5 vs. day 6–8 for other sera) than with HI-NHS, HI-FBS or FBS (Fig. 1), as quantitated by the $\Delta\Delta$Ct method and in a separate experiment by the infected cell standard curve. Compared with

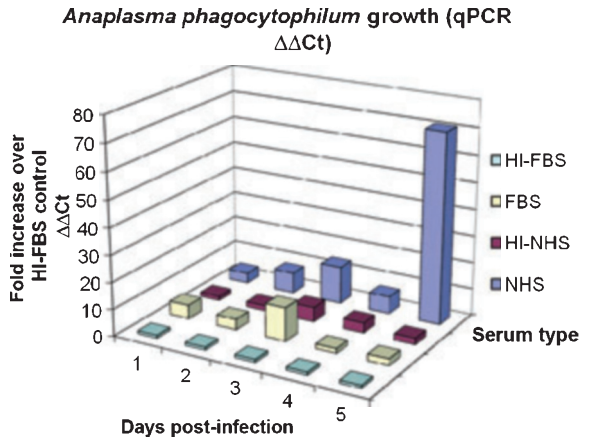

Fig. 1. Comparative growth of *Anaplasma phagocytophilum* in heat inactivated and complement-containing FBS and normal human serum (NHS). Note the rapid expansion of bacterial load when incubated in NHS at 5 days compared with heat-inactivated and other serum supplements.

HI-FBS, *msp2* content at d5 was 71-fold greater for NHS but only two-fold higher for HI-NHS and FBS. Despite the increased genomic content in NHS, morphologic quantitation of *A. phagocytophilum* infected HL-60 cells at day 5 revealed fewer infected cells with an increased number of morulae per infected cell in NHS (60%) and HI-NHS (54%) compared with FBS (100%) and HI-FBS (100%). Uninfected cell counts and *actB* content were similar among all cultures, demonstrating that HL-60 cells remained healthy and viable under these different conditions.

Anaplasma phagocytophilum survives and propagates better in human complement-containing medium *in vitro* than in FBS. The lower number of infected cells and the higher genomic content in NHS cultures suggest a facilitated adhesion or uptake mediated by complement. These observations underscore the pathogen's ability to avoid a heat-labile component, possibly complement-mediated killing, and suggest that *A. phagocytophilum* subverts complement to facilitate infection. Further study to determine whether complement is degraded, if C3 convertases are formed, and whether complement regulatory factors such as Factor H are utilised, will help elucidate the nature of *A. phagocytophilum*-complement cascade interactions or if the pathogen influences complement regulatory mechanisms to avoid killing and promote expansion and transmission.

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